

Management of anemia of chronic disease: beyond iron-only supplementation.

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Abstract

Chronic diseases are characterised by cell's autophagy and proteins disarrangement resulting in sarcopenia, hypoalbuminemia and hypo-haemoglobinaemia. Hypo-haemoglobinaemia causes worse prognosis independently of the principal disease.

Currently, the cornerstone of therapy of anaemia is iron supplementation, with or without erythropoietin for the stimulation of hematopoiesis. However, treatment strategies should incorporate the addition of heme, the principal biochemical constituent of haemoglobin.

Heme synthesis follows a complex biochemical pathway. The limiting step of heme synthesis is D-ALA availability which, for its synthesis, requires Glycine and Succinil-CoA. Consequently, treatment of anaemia should not be based only on iron availability, but also on the availability of the molecules fundamental for heme synthesis. Therefore, an adequate clinical therapeutic strategy should integrate the standard iron infusion and the supply of essential amino acids and vitamins involved in the heme synthesis.

We report preliminary data in selected elderly anaemic patients with congestive heart failure (CHF) and catabolic disarrangement, who, in addition to standard iron therapy, received personalized therapy with essential-AAs and vitamins involved in the maintenance of heme.

Notably, such individualized therapy resulted in a significant increase in the serum concentration of haemoglobin after 30 days of treatment compared to standard iron therapy.

Key words. Chronic diseases, iron deficiency, haemoglobin, anaemia, aminoacids, rehabilitation

1. Introduction

Non-communicable diseases account for 38 million deaths per year, according to the World Health Organization [1]. Of these deaths, chronic diseases (CD) constitute a major cause of mortality. The most common CD include congestive heart failure (CHF), senescence, cancer, chronic obstructive pulmonary disease (COPD), diabetes, arthritis, asthma, and some viral diseases such as hepatitis-C and acquired immunodeficiency syndrome [2].

All CD are characterised by a hypercatabolic syndrome due to low-grade inflammation (caused by specific molecules as cytokine, hormones, etc), which induces metabolic alterations as muscular and globular protein disarray. An increase in autophagy activity ensues, clinically resulting in sarcopenia, hypoalbuminemia and hypo-haemoglobinemia (otherwise known as anaemia) [3, 4]. Among the globular proteins, haemoglobin (Hb) is one of the most readily measureable in the blood.

Mounting experimental and clinical evidence demonstrate that anaemia and iron deficiency (ID) are present in patients with CD, resulting in significant limitation of therapeutic strategies rehabilitative such as rehabilitation programs, thereby worsening the prognosis of these patients [5–7]. Studies suggest there is the concomitant presence of inflammation with autophagy and subsequent protein disarrangement [8].

Gut dysbiosis, nutritional imbalance (malnutrition) with dysgeusia and, most importantly, ID with or without renal dysfunction capable of reduced erythropoietin-mediated erythropoiesis, are responsible for anaemia in CHF patients [5, 9]. Consequently, iron supplementation, with or without the addition of erythropoietin, is the most commonly recommended therapeutical approach to CHF-mediated anaemia [10].

Based on the current biochemical knowledge, the phenomenon of anaemia in CD (including CHF) should be considered in its entirety [11]. First of all, heme is the principal biochemical constituent of haemoglobin, with ID contributing partially to the anaemia in CD. Additional contributory factors in anaemia include the tetrapyrrolic proteic rings to which iron binds, thereby facilitating the metabolic function of heme, a hemoprotein. Further considerations include the production of D-Amino-levulinic acid (dALA) as the limiting step in the synthesis of the heme ring. D-Amino-levulinic acid (dALA) is derived from amino acid Glycine and Kreb's intermediate Succinyl-CoA [12].

Therefore, adequate treatment of anaemia in CD necessitates incorporation of standard iron infusion, along with supplementation of essential amino acids (EAAs) and vitamins involved in heme synthesis.

1.1 Iron and Heme

Iron (Fe), a requisite metal in almost all biological systems, is necessary for numerous critical processes such as DNA synthesis, heme and iron-sulfur cluster synthesis etc. Therefore, cellular regulation of iron concentration is essential for maintenance of normal physiology [13].

About 70% of body's iron is found in the red blood cells as haemoglobin, and in muscle cells as myoglobin. Iron, an essential element for blood production, is a crucial component of a very large class of metalloproteins containing heme, hence the name, hemoproteins.

Heme is an organic, ring-shaped molecules consisting of an iron ion coordinated to four pyrroles which are small pentagon-shaped molecules with 4 carbons and 1 nitrogen, which together form an iron-binding tetrapyrrole called porphyrin (**Figure 1**). Thus, heme is an iron-binding porphyrin [11]. Interestingly, the iron plays a balanced attractive force interacting with the nitrogen molecules of heme, thus, electrons stay balanced and the global molecule remains stable.

There are 4 different forms of heme in nature: heme-A, -B, -C and -O; they influence the function of the molecules in which heme is present. Although heme-B is the most common form, heme-A and -C are present in many molecules. Biochemical behaviors of the most common heme groups are regulated by differences of the functional groups in the side chains bound to carbon 3, 8 and 18 [11].

1.2. The synthesis of heme

Porphyrin synthesis, the biochemical pathway from which heme is derived, begins with the synthesis of D-Amino-levulinic acid (D-ALA) which is also the limiting step in heme synthesis [12].

D-ALA originates from amino acid (AA) glycine and from Krebs cycle intermediate *Succinyl-CoA* which comes from the *α-Ketoglutarate* or from the EAAs isoleucine, methionine, threonine or valine. Interestingly, the D-ALA synthesis occurs inside the mitochondria, facilitated by the enzyme named ALA-synthase which is negatively regulated by glucose and heme concentration. Importantly, enzyme inhibition is also dependent on the stability and availability of its mRNA in the mitochondria. Notably, AAs are the sole source of carbon and nitrogen atoms provided to D-ALA, demonstrating the narrow link between metabolism of AAs and energetic metabolism.

Released from the mitochondria, two D-ALA molecules are condensed to form *porphobilinogen* in the cytoplasm. The synthetic reaction continues until the formation of *coproporphyrinogen-III* which is transported inside the mitochondrial matrix and converted to

protoporphyrin-IX. The enzyme *ferrochelatase* then inserts the iron atom, forming heme which is shuttled in the cytoplasm where it is utilized in the synthesis of heme-based molecules [14]. The process of heme synthesis is illustrated in the **Figure 2**.

1.3. Functions of heme

Heme and hemoproteins have many biological functions. The presence of an iron atom serves as a source of electrons during electron transfer or redox chemistry, thereby giving heme the ability to transport diatomic gases and to exert chemical catalysis with electron transfer.

The hemoproteins participate in many diverse biological actions (such as oxygen transport) fundamental for life. Indeed, although haemoglobin and myoglobin are the two best known hemoproteins, other important, but often overlooked, enzymes which belong to hemoproteins include: cytochrome p450s, cytochrome-c oxidase, cyclooxygenase 2, catalase, peroxidases and endothelial nitric oxide synthase. In addition, as part of the electron transport chain, hemoproteins also enable electron transfer. A change in iron content affects important cell survival systems, illustrating that heme is not only important for oxygen transport, but also plays a fundamental role in other important metabolic pathways such as: energy production; transformation of many molecules and detoxification of aggressive molecules such as oxygen free radicals; regulation of inflammation and/or vascular tone; and blood coagulation [11].

1.4. Other molecules involved in heme synthesis

CD, especially if associated with qualitative malnutrition, induce a hyper-catabolic state and consequent protein disarrangement, which can precipitate the development of anaemia secondary to a reduction in hemoglobin. A schematic representation of this link is proposed in **Figure 3**. Independently from iron, other molecules which are strictly related to heme synthesis include:

VITAMIN B1. Its' pyrophosphate ester, thiamine diphosphate (TPP) is a co-factor for enzymes that catalyse alpha-keto acids of molecules involved in the Kreb's cycle and its intermediary metabolism [15].

VITAMIN B6. It co-catalyses reactions related to the anabolism and catabolism of AAs, facilitating the reactions of transamination. Interestingly, it is involved in protein folding, interacting with the folate cycle. In addition, vitamin B6 is a scavenger of free oxygen radicals [16].

VITAMIN B9 (Folate). It is a co-factor of many enzymes involved in the redox reactions and transfer of AAs' one-carbon unit (DNA methylation) [17].

VITAMIN D. It has anti-inflammatory properties, reducing circulating cytokines (IL-6, IL-1B) that counteract catabolism and autophagy. It stimulates the synthesis of anabolic molecules (as fibroblast growth factor-23 *alias* FGF-23) and increases red blood cell lifespan. In addition, it modulates hepcidin, a molecule responsible for the regulation of iron metabolism [18].

AMINO ACIDS. Hemoproteins (as haemoglobin), consisting of heme (as the metabolically active part) and surrounding proteins (as *globin* molecules), contain a large number of different AAs. Previous work has shown that administration of free EAAs mixtures tailored to the human metabolic process was able to improve anabolism, aerobic metabolism, and mitochondrial neogenesis [19, 20]. Rapidly absorbed, this mixture contains appropriate stoichiometric amount of all EAAs which can be converted into non-essential-AAs (NEAAs) as Glycine [21]. Moreover, the EAAs mixture contains: *a*) L-Lucine, which modulates the enzyme mTORC1 involved in haemoglobin production, and *b*) Histidine, which stabilises bound O₂ and acts as a gate allowing ligand entry of both haemoglobin subunits [22, 23]. Histidine, essential in globin synthesis and erythropoiesis,-has also been implicated in the enhancement of iron absorption from human diets. Furthermore, histidine has already proven to be effective both in improving anti-anaemic efficiency and limiting the damages resulting from iron overload and oxidative stress caused in CKD [24]. Conversely, beta-alanine supplementation would impair protein synthesis by reducing histidine concentration and availability [25].

In light of these considerations, provision of the molecules involved in the-synthesis of heme and hemoglobin is essential, even more so if patients are nutritionally deficient.

2. Methods

Based on the aforementioned fundamental biochemical knowledge and in observation of “*good medical practice*” (www.gmc-uk.org), we conducted a controlled clinical trial which integrated personalised standard therapy with iron infusion, along with the administration of specialized mixtures rich in free EAAs [21] and vitamins (B1, B6, B9, D) to treat heme synthesis deficiency in a cohort of select elderly female patients (n=15; age 78.3 ±8.5 y.o.) with CHF. Written informed consent was obtained; ethical approval was not required under local legislation. The inclusion criteria were: 1) anemia (Hb >8.5/<11.5 mg/dl); 2) symptoms and signs of stable CHF for at least 3 months on a standard medical therapy with beta-blocker, diuretics, ACE-inhibitor or ARB; 3) protein disarrangement (albuminemia <3.5 g/dl), but normal BMI (>24); 4) iron deficiency (plasma iron <50 mg/dl, ferritin <100 mg/dl, or serum ferritin within range 100-299 mg/dl when transferrin

saturation is <20%); 5) inflammation (by CRP >5 mg/l); and 6) vitamin D and/or folate lower than the normal ranges (15.2-90.1 pg/ml and >3.00 ng/ml respectively).

Since these patients, as well as the haemoglobin concentration, had levels of albumin and vitamins below the minimum, according to good medical practice, we treated them for 30 days with daily intravenous administration of 100 mg of ferric carboxymaltose, integrated with oral administration of 4g of specific free AAs mixture rich in essential ones (84%), 15mg of Vit.B9, 0.15mg of Vit.B1 and Vit.B6 and 1000UI/die of Cholecalciferol (Vit.D).

Intravenous ferric carboxymaltose has been previously demonstrated to improve symptoms, functional capacity, and quality of life in HF patients, even in the absence of anemia, in the FAIR-HF clinical trial [10]. Subsequent clinical trials have re-confirmed the benefits of intravenous ferric carboxymaltose, with improvement in exercise capacity in the EFFECT-HF trial [26], and reduced risk of hospitalizations for HF exacerbation in the CONFIRM-HF trial [27], and in the recently published AFFIRM-AHF clinical trial [28].

The control group consisted of a cohort of elderly female patients (n=15; age 76.1 ± 11 y.o.) with the same inclusion criteria, except that the levels of albumin and vitamins were near the lower limits of normal range. This group received only standard iron therapy without supplementation. The baseline mean clinical biochemical data from two cohorts are summarized in **Table 1**.

3. Results

Baseline and post-intervention clinical biochemical data are summarized in **Table 2**. Increased levels of sideremia, ferritin, saturated transferrin were observed in both groups; there was no difference in total transferrin values. However, only the experimental group receiving intravenous iron therapy *PLUS* integrated therapy demonstrated increased levels of haemoglobin (**Figure 4A-B**) compared to the standard iron therapy only group [see **Table 3** and **Figure 4C-D**].

The Student *t*-Test was used to compare the data before and after treatment, with p-value <0.05 considered significant.

4. Discussion and Conclusion

The incidence of anemia (32%) is common in HF patients, with concurrent iron and folate deficiencies noted in 43% of anemic patients, compared to 15% of non-anemic patients [29]. Currently, the standard therapy of anemia is primarily based on the supplementation of iron, with or without erythropoietin for hematopoiesis stimulation. Previous randomized, controlled studies with intravenous iron in HF patients reported that haemoglobin increased after 4 or 6 months [7, 10, 30, 31]. Indeed, if a deficiency of fundamental molecules (such as amino acids and vitamins) results in the lack of heme synthesis, iron supplementation alone will not lead to a proportional hemoprotein increase, or Hb *in primis*. In addition, an isolated increase in iron, without any accompanying augmentation in heme, could favor the persistence of oxidative stress (via Fenton/Haber-Weiss reactions), chronic inflammation and autophagy [32]. The mode of iron supplementation also appears to be important, as oral supplementation has not been shown to be effective in improving exercise capacity in patients with HF with reduced ejection fraction and iron deficiency [33].

Based on these preliminary data demonstrating a rapid escalation in hemoglobin level (within 30 days after interventions aimed at increasing iron and heme), we conclude that the effective approach to treating heme synthesis (including anaemia) in CD must consider not only the iron availability, but also integrate a therapeutic strategy which counteracts catabolism. Therefore, the standard intravenous or oral iron supplementation should incorporate the supply of specific mixtures of EAAs and vitamins involved in biochemical pathway of the heme synthesis as illustrated in the **Figure 5**. Consequently, the careful evaluation of nutritional status of patients, the presence of catabolism and of molecules involved in heme synthesis, as well as their integration, must therefore be the first step of the personalized therapeutic intervention aimed at correcting the state

of anaemia in patient with CD such as CHF. Our therapeutical approach based on biochemical data should be confirmed in a large-scale clinical trial.

Main messages

- In chronic hypercatabolic diseases (such as CHF), the metabolism of iron and heme-protein is markedly impaired, inducing anaemia and likely impairment of many other hemoproteins involved in essential metabolic pathways.
- Heme is the metabolically active part of haemoglobin. It is characterised by the presence of iron atoms linked to tetrapyrrole groups.
- Many other important biologically active molecules named hemoproteins (including Hb) contain heme as the metabolically active part, with surrounding proteins (as *globin* molecules) containing a large number of different amino acids.
- Maintenance of an adequate blood concentration of both iron and heme is fundamental for proper function of the heme-containing enzymes.
- In patients with anemia and chronic hypercatabolic diseases, the correction of deficiencies in iron, as well as metabolic substrates required for all hemoproteins, is essential for proper treatment.

Conflict of Interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Table 1. Comparison of baseline antropometric and clinical biochemical data from patients who have received integrated therapy and iron standard therapy (control). Note that patients that received iron standard therapy have nutritional parameters (albumin and vitamins) close to the lower limit of normal range.

	INTEGRATED THERAPY (n = 15)	STANDARD THERAPY (n = 15)	Normal value
Age (y.o)	78.3 ±8.5	76.1 ±11	---
BMI	26.9 ±1.85	25.5±1.91	<25
Haemoglobin (g/dl)	10.2 ±0.8	10.37 ±0.91	>11.5
Creatinine (mg/dl)	1.06 ±0.25	1.02 ±0.19	0,5-1,1
Albumin (g/dl)	3.31 ±0.37	3.55 ±0.08	>3.5
Iron (%)	44.33 ±8.56	45.87 ±12.10	55-65
Ferritin (mg/ml)	73.73 ±38.81	94.80 ±40.76	5-204
Sideremia (ug/ml)	36.07 ±6.85	33.72 ±9.33	50-170
Transferrin saturated (%)	14.33 ±4.24	12.88 ±2.18	20-45
Transferrin total (mg/dl)	228.53 ±48.09	224.33 ±50.0	180-380
Vit. B9 (ng/ml)	2.32 ±0.35	3.13 ±0.20	3
1,25-OH Vit.D (pg/ml)	17.73 ±4.23	21.87 ±2.25	21-100
RCP (mg/L)	10.67 ±2.43	10.82 ±2.70	<5
NT-proBNP (pg/ml)	2449.2 ±2048.69	2650.27 ±2177.65	<450
LVEF (%)	54 ±6	56 ±5	>50
CVP (mmHg)	5.5±1.08	5.73 ±1.05	<8

Table 2. Clinical biochemical data before (baseline) and after (30 days) integrated therapy. *

p<0.01

INTEGRATED THERAPY	BASELINE	30 DAYS	t	p
Haemoglobin (g/dl)	10.1 ±0.76	11.06 ±0.83 *	3.304	0.003
Ferritin (mg/ml)	73.73 ±38.81	390.93 ±196.4 *	6.147	0.000
Sideremia (μg /ml)	36.07 ±6.85	81.93 ±16.84 *	9.770	0.000
Transferrin saturated (%)	14.33 ±4.24	51.28 ±10.24 *	12.912	0.000
Transferrin total (mg/dl)	228.53 ±48.09	253.0 ±50.37	1.361	0.184

Table 3. Clinical biochemical data before (baseline) and after (30 days) standard iron therapy. *

p<0.01

STANDARD THERAPY	BASELINE	30 DAYS	t	p
Haemoglobin (g/dl)	10.37 ±0.91	10.66 ±0.93	0.863	0.395
Ferritin (mg/ml)	94.80 ±40.76	631.47 ±289.66 *	7.106	0.000
Sideremia (μg /ml)	33.72 ±9.33	109.0 ±28.02 *	9.872	0.000
Transferrin saturated (%)	12.88 ±2.18	56.23 ±9.85 *	16.642	0.000
Transferrin total (mg/dl)	224.33 ±50.0	218.33 ±55.74	0.310	0.759

Figure legends

Figure 1. Haemoglobin structure.

Figure 2. Heme synthesis and degradation pathways.

Figure 3. Schematic representation of the effect of chronic diseases and malnutrition on the onset of anaemia.

Figure 4. A and B) Change in hemoglobin concentration in each patient consequent to integrated therapy. Histogram shows the mean (\pm sd) concentration of haemoglobin before (pre) and after (post) integrated therapy. **C and D)** Change in hemoglobin concentration in each patient consequent to iron standard therapy. Histogram shows the mean (\pm sd) concentration of haemoglobin before and after iron standard therapy. The black line indicates the minimum reference value. * $p<0.01$.

Figure 5. Schematic representation of the effects of integrated therapy on the containment of anemia.